diseases. We have characterized the lipid-induced aggregation of tau by FCS. We find that aggregation occurs when the amount of protein bound to the lipid bilayer exceeds a critical surface density. Our results suggest that the lipid bilayer facilitates protein-protein interactions both by screening charges on the protein as well as by increasing the local protein concentration, resulting in rapid aggregation. Our work highlights the versatility of FCS in studying this important class of proteins.

98-Symp
Total Internal Reflection with Fluorescence Correlation Spectroscopy
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The combination of total internal reflection illumination with fluorescence correlation spectroscopy (TIR-FCS) allows one to examine in quantitative detail a variety of biophysical properties related to the motions and interactions of fluorescent molecules near the interface of a transparent planar surface and an adjacent solution. Several experimental and theoretical aspects of this combination will be discussed. TIR-FCS has allowed characterization of local diffusion coefficients and concentrations of fluorescently labeled antibodies in solution but very close to substrate-supported phospholipid bilayers. TIR-FCS has also been used to examine the interaction kinetics of fluorescently labeled mouse IgG specifically and reversibly associating with the mouse receptor Fc-gamma-RII, which was purified and reconstituted into substrate-supported planar membranes. This method also has the potential, through the use of a single fluorescent reporter, of providing information about the thermodynamics/kinetics of nonfluorescent molecules which participate in surface binding mechanisms; e.g., those that compete with fluorescent reporters for surface-immobilized receptors or those that interact on the surface with the receptors and reduce or enhance the interaction of the fluorescent reporters with the surface binding sites.

99-Symp
Measuring Biomolecular Interactions with Single Wavelength Fluorescence Cross-Correlation Spectroscopy
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Biomolecular interactions are strongly influenced by the complex cellular environment in which molecules will interact not only with each other but often compete with or interact via other molecules. Therefore it is necessary to establish methods which can quantitatively measure interactions within the environment of cells and organisms. Fluorescence Cross-Correlation Spectroscopy (FCCS) is a widely used tool for this purpose. FCCS determines interactions by exciting two fluorescently tagged molecules with different excitation and emission spectra. It uses two distinct laser lines, which have to be brought to the same focus, for excitation. But in tissues, where aberrations can be significant, the overlap of the focal volumes can be strongly position and time dependent rendering quantification difficult. In single wavelength FCCS (SW-FCCS) a single laser line is used to excite two fluorophores with similar excitation but distinct emission spectra making the alignment of two lasers unnecessary. SW-FCCS has been shown to work with quantum dots, tandem dyes, and organic fluorophores. But in particular it can be applied to fluorescent protein pairs, e.g. GFP and mRFP/mCherry, which facilitates its application in live samples. Since its inception SW-FCCS has been applied in live cells and organisms to determine receptor dimerization, monitor phosphorylation of activated receptors, and determine biomolecular affinities. We will discuss advantages and pitfalls of SW-FCCS and will show its extension to an imaging format. Confocal SW-FCCS can measure interactions only at single spots. A more accurate picture of the interactions happening simultaneously in a cell can be obtained by performing SW-FCCS in an imaging format where hundreds of points can be measured simultaneously. We therefore extended SW-FCCS to camera based FCCS to measure interactions at multiple points in a cell.

100-Symp
40 Years of FCS - A Success Story in Biology
Petra Schwille.
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Four decades after its invention, Fluorescence Correlation Spectroscopy has found its way into the canon of standard methods for the quantitative analysis of biological systems. Originally designed for the study of fast molecular dynamics modulating the fluorescence emission of molecules, and for a long time being exclusively applied in well-equilibrated buffer solutions, a large body of instrumental and methodological improvements have paved the way for its rigorous aplication in the study of complex living systems. After briefly reviewing some of the most important milestones in FCS development, I will discuss challenges and prospects for its potential future use in systems biology.

Minisymposium: Allosteric Communication in Ring-shaped ATPases

101-MiniSymp
Lis1 Uncouples Allosteric Communication Between Dynein's AAA+ Motor and Microtubule Binding Domains
Samara L. Reck-Peterson[1], Julie Huang[1], Anthony J. Roberts[1], Andres E. Leschziner[2].
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Cytoplasmic dynein, the large microtubule-based motor protein, is highly regulated in cells, enabling it to be deployed to specific sites, collect cargos, and translocate them towards the microtubule minus-end at specific times. Defects in the regulation of dynein can cause neurological diseases; for example, mutations in the dynein co-factor Lis1 cause lissencephaly. Dynein's ''engine'' is evolved from ring-shaped AAA+ ATPases, and its microtubule-binding domain lies at the tip of a coiled-coil stalk, however how these elements might be acted upon to achieve control remains unknown. Here, using purified proteins from S. cerevisiae, we dissect how Lis1/Pac1 and its binding partner Nudel/Ndl1 control cytoplasmic dynein. At the single molecule level, we find that Lis1 slows dynein's velocity and prolongs its microtubule encounters. Nudel allows Lis1 to function at lower concentrations, suggesting it acts as a tether between Lis1 and dynein. High precision analysis shows that Lis1 causes dynein to adopt a microtubule-anchored state by uncoupling dynein's ATPase cycle from its binding to microtubules, such that ATP is consumed without the usual microtubule release and forward motion. To understand the structural basis for Lis1 regulation, we mapped its binding site on dynein using electron microscopy and 2D image analysis. Unexpectedly, we find that Lis1 binds at the interface between dynein's AAA+ ring and microtubule-binding stalk. Mutational analyses are suggestive of electrostatic interactions at this dynein-Lis1 interface, which are disrupted by lissencephaly-causing mutations. We propose that Lis1 regulates the transmission of structural changes between dynein's AAA+ ring and microtubule binding stalk, biasing dynein towards a microtubule-anchored state. In vivo this could allow Lis1 to assist in dynein's microtubule plus-end localization and cargo loading, and prevent slippage during dynein's tension-bearing roles.

102-MiniSymp
Separable Roles for ATPase Domains Involved in Virulence Factor Secretion
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Pathogenic bacteria utilize specialized protein secretion machinery in order to either affect their host cells or to alter the bacterial cell surface itself. One such emerging secretion system, originally described in Mycobacterium tuberculosis, has been termed the Type VII secretion system (T7S) in bacteria. This system is defined in part by the presence of membrane-associated FtsK/SpoIIIE-like AAA-ATPase domain containing proteins, each containing multiple active sites. While presumed to be involved in the secretion of the virulence proteins, the function of the ATPases has not been previously determined. Furthermore, the broader family of these secretion systems includes gene clusters where the FtsK/SpoIIIE-like protein is split and coded by two genes rather than one. Here we explore the role of these ATPases in protein secretion using two T7S systems, one with a single ATPase and the other with a naturally split ATPase. Using complementary biochemical and in vivo assays, we demonstrate that the ATPases are essential for protein secretion. Further, we find that the active sites contribute differentially to substrate interaction and export, thus suggesting a novel system in which a subset of the ATPase domains may function as their own adapter molecules.

103-MiniSymp
Allosteric Regulation of Nucleotide Binding to the Proteasomal ATPases
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Protein degradation by the eukaryotic 26S proteasome or the homologous archaeal PAN-20S proteasome complex is a multistep process that requires ATP hydrolysis by the proteasome-associated AAA ATPase complex. However, the mechanisms by which these hexameric ATPases bind and hydrolyze ATP to promote protein breakdown are poorly understood. Although